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Comparative Antioxidant, Phytochemical and Proximate Analysis of Aqueous and Methanolic Extracts of *Vernonia amygdalina* and *Talinum triangulare*

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Abstract: This study evaluated and compared *in vitro* antioxidant activity, phytochemical constituent and proximate analysis of aqueous and methanolic leaf extracts of *Vernonia amygdalina* and *Talinum triangulare* to determine their acceptability in folkloric medicine. The total phenolic content of aqueous and methanolic extracts of *V. amygdalina* (0.822±0.050 and 0.681±0.050 mg gallic acid equivalent GAE/g respectively) was higher than aqueous and methanolic extract of *T. triangulare* (0.456±0.040 and 0.288±0.020 mg GAE/g respectively). Furthermore, DPPH scavenging activity of *V. amygdalina* was significantly higher ($p<0.05$) than *T. triangulare* at all levels of concentration (100, 200 and 300 µg/ml). Lipid peroxidation was inhibited by all samples, although there was no significant difference ($p>0.05$). Aqueous extracts of leaf tested positive to tannin, phlobatannins, cardiac glycosides, saponins, phenols, flavonoids and alkaloids. The proximate composition of *V. amygdalina* leaf showed higher percentage crude fibre, fat, protein and total carbohydrate content than *T. triangulare* except moisture and ash contents. Methanolic and aqueous leaf extracts of *V. amygdalina* possess higher antioxidant properties, phyto-nutrients and longer shelf life than *T. triangulare* and hence it's pervading use and acceptability in folkloric and trado-medicine.

Key words: Aqueous and methanolic leaf extracts, *Vernonia amygdalina, Talinum triangulare*

INTRODUCTION

Antioxidants have been known to play protective role in human body against deleterious effects of reactive free radicals and it has been defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990). They are chemical compounds that can prevent, stop, or reduce reactive effect of free radicals. These effects include oxidative damage to membranes and enhanced susceptibility to lipid peroxidation or enzyme inactivation (Farombi and Fakoya, 2005; Sathishsekar and Subramanian, 2005). Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron, by cleavage of a radical to give another radical and also via redox reactions (Halliwell and Gutteridge, 2008; Bahorun *et al*., 2006). Free radicals include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), peroxyl ($ROO^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$). Also, hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH), are not free radicals but generally, they are called oxidants, although, they can easily lead to free radical reactions in living organisms (Genestra, 2007). Biological free radicals are thus highly unstable molecules that have electrons available to react with various organic substrates such as lipids, proteins, DNA and eventually progress to oxidative stress. Thus, antioxidant containing foods like fruits and vegetables could have strong protective effect against the risk of major diseases such as cancer and cardiovascular diseases (Kaur and Kapoor, 2001; Amic *et al*., 2003). Vegetables and fruits extracts are often employed in folkloric medicine to treat several ailments (Wang *et al*., 2007).

Vernonia amygdalina Del. (Asteraceae) commonly called bitter leaf contains anti-nutritional factors such as alkaloids, saponins, tannins and glycosides responsible for its bitter taste (Buttler and Bailey, 1973; Ologunde *et al*., 1992). It is consumed as a vegetable in Nigeria and many areas of East Africa (Mensah *et al*., 2008). However, extract of bitter leaf had been reported to exert antibiotic action against drug resistant microorganisms and possesses antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities (Akinpelu, 1999; Dahanukar *et al*., 2000). Furthermore, the root provides one of the commonly used chew sticks in Nigeria due to alleged beneficial effect on dental caries (Aregheore *et al*., 1998). The leaves and bark in Ethiopian local medicine are used as purgative, against menstrual pain and wound dressing (Akah and Okafor, 1992; Uhegbu and Ogbuchi, 2004).

Talinum triangulare (Jacq.) Wild (Portulacaceae) is a herb with fleshy green leaves, succulent stem and pink

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flowers which are rarely white (Keay, 1981). It is an all season vegetable, grown mostly in West Africa from seed or by vegetative propagation (Akobundu and Agyakwa, 1998; Imoh and Julia, 2000). *T. triangulare* leaf is used in folkloric medicine to treat diuretic and gastro-intestinal disorders (Mensah *et al*., 2008) and oedema. Furthermore, the leaves serve as sauce, condiment, spice or flavorings in foods (Mbang *et al*., 2008). Therefore, this study was designed to compare the antioxidant properties, phytochemical and proximate composition of *V. amygdalina* and *T. triangulare* in order to gain insight into their acceptability in therapeutic and folkloric use.

MATERIALS AND METHODS

Chemicals: DPPH (2,2 diphenyl-1- picrylhydrazyl hydrate), gallic acid, ascorbic acid and Folin-Ciocalteu's reagent were purchased from Sigma Aldrich, USA. All other chemicals and reagents used were of analytical grade.

Collection of plant materials: *Vernonia amygdalina* and *T. triangulare* were collected from a farmland at Ilisan Remo, Ogun State, South-Western Nigeria. Both plants were authenticated by Prof. Edward B. Esan, a plant scientist in the Department of Chemical and Environmental Sciences, Babcock University, Ogun State, Nigeria.

Extraction of plant material: Fresh leaves of *V. amygdalina* and *T. triangulare* were air dried and then ground to fine powder. The pulverized samples (20 g) were soaked in 150 ml of 100 % methanol and 300 ml of distilled water for 72 h before extraction. The methanolic and/or aqueous extracts were concentrated to dryness in a rotary evaporator and thereafter preserved in a refrigerator at 4^{o}C until further use.

Determination of total phenolic content: The total phenolic content was estimated as described by Singleton and Rossi (1965) and modified by Gulcin *et al*. (2003). One ml aliquot of extracts or standard solution of gallic acid (10, 20, 30, 40 and 50 mg/l) was added in a volumetric flask containing 9 ml of water. One ml of Folin-Ciocalteu's reagent was added to the mixture and vortexed. After 5 min, 10 ml of 7% sodium carbonate was added to the mixture and incubated for 90 min at 25^{o}C. The absorbance against reagent blank was determined at 750 nm. A reagent blank was prepared and the amount of phenolic compound in the extract was determined from the standard curve. The total phenolic content of the plant was then calculated as shown in the equation below and expressed as mg Gallic Acid Equivalent (GAE)/g fresh weight. All samples were analyzed in duplicates.

$$C = c. m/V$$

Where:

C = Total content of phenolic compound in gallic acid equivalent (GAE)/g
c = The concentration of gallic acid established from the calibration curve (µg/ml)
V = Volume of extract (ml)
m = Weight of the crude plant extract (g)

Antioxidant assay: Rapid Thin Layer Chromatography (TLC) screening for antioxidant activity was carried out by spotting a concentrated methanolic solution of the extract on silica gel plates. The plates were developed in methanol: ethyl acetate (2:1, v/v) afterwards air-dried and sprayed with 0.2% w/v DPPH spray in methanol. The plates were visualized for the presence of yellow spots. The radical scavenging activity of leaf extracts was performed according to the DPPH spectrophotometric method of Mensor *et al*. (2001). One ml of a 0.3 mM DPPH methanol solution was added to a 2.5 ml solution of the extract or standard (100 µg/ml, 200 µg/ml, 300 µg/ml) and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage Antioxidant Activity (AA %), using the formula:

$$AA\% = [1-(Abs_{sample}/Abs_{control})] \times 100$$

Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control. Solutions of ascorbic acid and gallic acid served as positive controls. This assay was carried out in triplicates for each concentration.

Inhibition of lipid peroxidation: A modified Thiobarbituric Acid Reactive Substances (TBARS) assay was used to measure the lipid peroxide formed using egg yolk homogenate as lipid-rich media (Ruberto and Baratta, 2000). Egg homogenate (0.5 ml, 10 % v/v) was added to 0.1 ml of extract (1 mg/ml) and the volume made up to 1 ml with distilled water. Thereafter, 0.05 ml of $FeSO_4$ was added and the mixture incubated for 30 min. 1.5 ml of acetic acid was then added followed by 1.5 ml of TBA in SDS. The resulting mixture was vortexed and heated at 95^{o}C for 60 min. After cooling, 5 ml of butan-1-ol was added and the mixture centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm and converted to percentage inhibition using the formula:

$$(1 - E / C) \times 100$$

Where:

C = Absorbance of fully oxidized control and
E = Absorbance in the presence of extract

Phytochemical screening: Chemical tests were carried out on the aqueous and methanolic extracts for the

qualitative determination of phytochemical constituents using standard procedures as described by Harbone (1973) and Sofowora (1993).

Proximate analysis: The chemical tests were carried out on the plant samples for the quantitative determination of physico-chemical constituent using standard procedures as described by Pearson (1976).

Statistical analysis: This was done with the aid of Windows Microsoft Excel and SPSS for windows; SPSS Inc., Chicago, standard version 14.0 to determine differences between means using Analysis of Variance (ANOVA). Data were reported as Mean±Standard deviation.

RESULTS

The total phenolic content of crude extracts was obtained from the regression equation for the calibration curve of standard gallic acid ($y = 0.001x + 0.007$, $R^2 = 0.996$) and expressed as Gallic Acid Equivalent (GAE). The GAE indicated that aqueous and methanolic extracts of *V. amygdalina* had higher phenolic content than those of *T. triangulare* (Table 1).

Rapid TLC screening for antioxidant activity in the plant extracts was positive. The percentage DPPH radical scavenging activity of the extracts showed that methanolic extracts of *V. amygdalina* and *T. triangulare* had high antioxidant activity than aqueous extract of *V. amygdalina* and *T. triangulare* respectively. However, the antioxidant activity of aqueous and methanolic extracts of *V. amygdalina* was slightly higher than those of aqueous and methanolic extract of *T. triangulare.* Nevertheless, the antioxidant activity of both *V. amygdalina* and *T. triangulare* extracts was low compared with standard gallic and ascorbic acid (Fig. 1-3).

Percentage inhibition of lipid peroxidation by extracts tested showed no significant difference ($p>0.05$) (Table 1).

Phytochemical analysis of extracts tested positive to tannins, saponins, flavonoids, phenols and alkaloids, excluding methanolic extract of *T. triangulare* that tested negative for tannin. Only the aqueous extracts tested positive for tannins, phlobatannins and cardiac glycosides. However, all extracts tested negative for terpenoids, cardenolides and anthraquinones (Table 2).

The proximate composition of *V. amygdalina* leaf indicates higher percentage of crude fibre, crude fat, crude protein, and carbohydrate than *T. triangulare* except the moisture and ash contents (Table 3).

DISCUSSION

Recently, there have been increased scientific interests in the study of antioxidants, particularly those intended to prevent the presumed deleterious effects of free radicals

Table 1: Quantitative determination percentage inhibition of lipid peroxidation and total phenol content of aqueous and methanol extracts of *V. amygdalina* and *T. triangulare*

Sample	Concentration (µg/ml)	Percentage Inhibition of lipid peroxidation	Total phenol (mg/g GAE)
Aqueous extract of *V. amygdalina*	100	72.85±0.495*	0.822±0.05
Methanolic extract of *V. amygdalina*	100	72.40±0.424	0.681±0.05
Aqueous extract of *T. triangulare*	100	72.25±0.919	0.456±0.04
Methanolic extract of *T. triangulare*	100	73.85±0.636	0.288±0.02

*Indicates mean±standard deviation

Table 2: Phytochemical constituent of aqueous and methanolic extracts of *Vernonia amygdalina* and *Talinum triangulare* leaves

	V. amygdalina		*T. triangulare*	
Phytochemical	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
Terpenoids	-	-	-	-
Tannins	++	+	+	-
Saponins	++	++	++	+
Phlobatannins	++	-	++	-
Cardiac glycoside	++	-	++	-
Flavonoids	++	++	++	++
Cardenolides	-	-	-	-
Anthraquinones	-	-	-	-
Phenol	+	+	+	+
Alkaloid	++	++	+	+

+ = Indicates trace; ++ = Indicates abundant; - = Indicates absent

Table 3: Proximate compositions of *Vernonia amygdalina and Talinum triangulare*

Plant	Moisture (%)	Fiber (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate (%)
V. amygdalina	18.6±0.6	13.8±0.06	14.9±0.2	4.56±0.4	21.6±0.14	26.5±0.6
T. triangulare	23.1±0.2	12.5±0.04	9.4±0.1	1.88±0.2	47.6±0.07	5.52±0.4

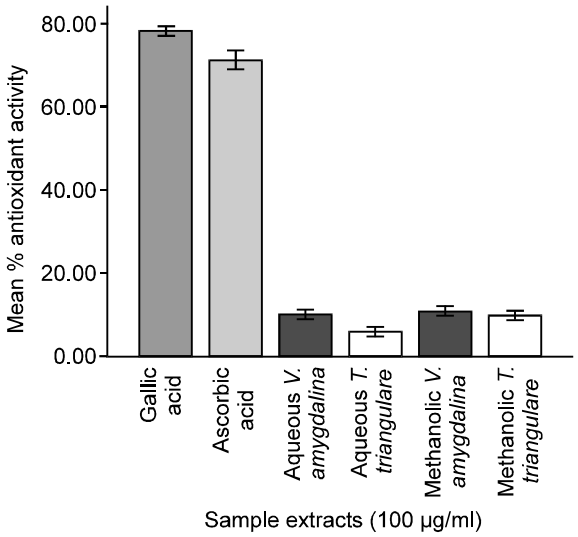

Fig. 1: Percentage scavenging activity of DPPH against 100 µg/ml sample extracts

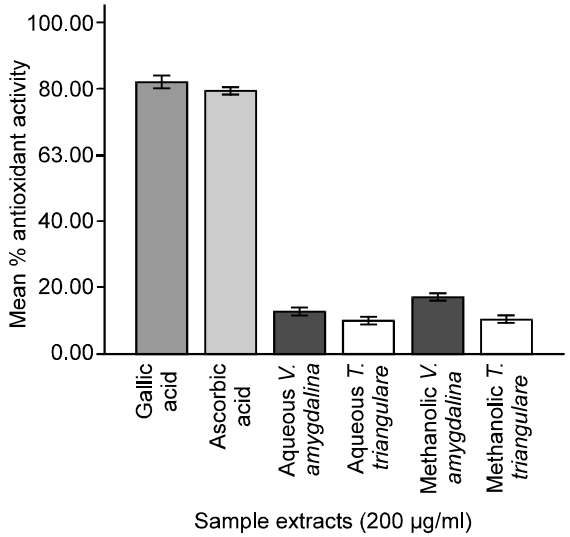

Fig. 2: Percentage scavenging activity of DPPH against 200 µg/ml sample extracts

in human body and deterioration of fats and other constituents of foodstuff. In both cases, antioxidants from natural rather than synthetic sources are preferred (Abdalla and Roozen, 1999).

The result demonstrated that the aqueous extract of *V. amygdalina* showed higher phenolic content than other extracts. Plant phenolics are major group of compounds acting as primary antioxidants or free radical scavengers (Kahkonen *et al*., 1999). All sample extracts tested positive using the rapid Thin Layer Chromatography (TLC) screening for the presence of antioxidant activity. The change in color of DPPH spray from deep violet to yellowish spots suggests presence of free radical scavengers. Furthermore, the free radical scavenging

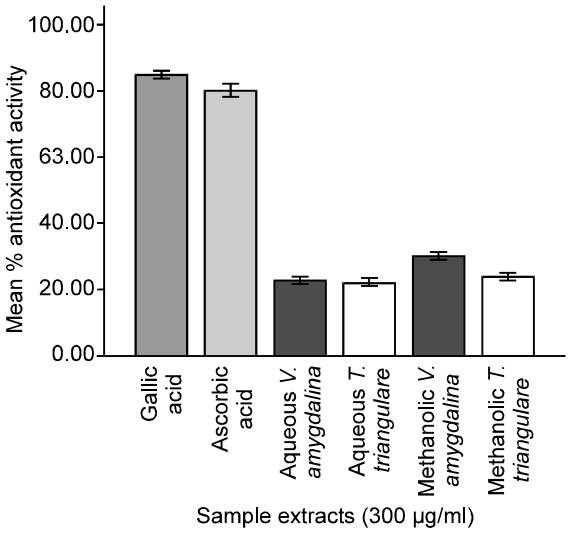

Fig. 3: Percentage scavenging activity of DPPH against 300 µg/ml sample extracts

power of the leave extracts of *V. amygdalina* and *T. triangulare* increased with increasing concentration of the extracts as evident in the rapid reduction of the stable DPPH radical and thus, may be considered good sources of antioxidants for nutrition, medicine and commercial use (Ayoola *et al*., 2008). Lipid peroxidation was prevented by 100 µg/ml extracts of both *V. amygdalina* and *T. triangulare*. Reports have shown that the oxidation index is a good indicator of degradation and lack of an antioxidant property. This result however, indicates inhibition of lipid peroxidation by leave extracts in the biological system and it is in close agreement with the work of Odukoya *et al*. (2006) that reported the antiperoxidative effect of leaves of *V. amygdalina* using the linoleic acid model system.

Phytochemical composition in leave extracts of *V. amygdalina* and *T. triangulare* include bioactive compounds such as saponins, phenols, flavonoids, alkaloids, phlobatannins, cardiac glycosides and tannins. This suggests stimulatory, antiseptic, anti-inflammatory and mild anti-hypertensive properties of the leave extracts (Ayoola *et al*., 2008; Mensah *et al*., 2008).

Proximate analysis result revealed that *V. amygdalina* leaf had higher total carbohydrate, crude fat, crude protein and crude fibre contents than *T. triangulare.* This suggests that *V. amygdalina* rather than *T. triangulare* could serve as a better source of dietary carbohydrate, protein and lipids. Hence, *V. amygdalina* adds to the calorific value of food and possesses odour and flavor carrying ability thereby enhancing the palatability of food. Mensah *et al*. (2008) also reported that crude fibre adds bulk to the food and prevents the intake of excess starchy food. However, *T. triangulare* had higher ash and moisture content than *V. amygdalina* leaf suggesting the

presence of high total inorganic residue, an indicator of the mineral content. High moisture content reduces the shelf life of food substances (Ruberto and Baratta, 2000). Hence, *V. amygdalina* leaf could have a higher shelf life than *T. triangulare* leaf.

Conclusion: Aqueous leaf extracts of *V. amygdalina* and *T. triangulare* contain more phytochemicals (tannins, phlobatannins and cardiac glycosides) in addition to methanolic extracts' saponins, phenols, flavonoids and alkaloids. The methanolic and aqueous leaf extracts of *V. amygdalina* possess higher antioxidant properties, phyto-nutrients and longer shelf life than *T. triangulare*. This may explain the wide acceptability and uniqueness of *V. amygdalina* in folkloric medicine to prevent or slow down the progress of various oxidative stress-related diseases.

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